

ACTN3 and AMPD1 Polymorphism and Genotype Combinations in Bulgarian Athletes Performing Wingate Test

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Abstract: The aim of the study was to investigate *ACTN3* (α -actinin-3) and *AMPD1* (adenosine monophosphate deaminase) polymorphism and genotype combinations in Bulgarian athletes competing in various sports and the relation to peak power output. A mixed group of athletes ($n = 52$) competing at national and international level and a matching genetic control group ($n = 109$) of volunteers were recruited. Participants were genotyped for *ACTN3* and *AMPD1* by polymerase chain reaction. There were no significant differences in *ACTN3* genotype distribution between athletes performing Wingate test (38% RR, 46% RX, 16% XX) and controls (41.2% RR, 46% RX, 12.8% XX). *AMPD1* distribution was (73% CC, 27% CT, 0% TT) and in controls (73.2% CC, 25% CT, 1.8% TT). Athletes performing Wingate test showed equal 33% frequency of RR/CC and RX/CC combination, and 12% RX/CT. Significantly higher ($P < 0.05$) peak power output (11.10 W kg^{-1}) was found in athletes with RX/CT combination compared to other combinations (range: $8.83\text{-}9.71 \text{ W kg}^{-1}$) and in R-power (RR + RX) and C-power (CC + CT) dominant models (9.91 W kg^{-1}). Mean power was higher ($P < 0.05$) in RX/CT combination (8.93 W kg^{-1}) compared to RR/CC (7.75 W kg^{-1}) and RR/CT (7.95 W kg^{-1}). In conclusion, the low frequency of T *AMPD1* allele in Bulgarian athletes might indicate that this mutant allele is related to the physical performance. The prevalence of R *ACTN3* and C *AMPD1* alleles suggests that they could contribute to anaerobic performance. Higher peak power in Wingate test is associated with RX/CT genotype combination and R- and C-power dominant models.

Key words: Genetics, metabolism, exercise, performance, peak power.

1. Introduction

Athletic performance in various sports is influenced by genetic traits and phenotype characteristics and is associated with the presence of certain gene polymorphisms [1-10].

The polymorphism of *ACTN3* (α -actinin-3) and *AMPD1* (adenosine monophosphate deaminase) genes have been extensively studied in athletes [11-17]. In both genes, the polymorphism is due to single base mutations [18]. The *ACTN3* gene encodes the α -actinin protein in human skeletal muscle. Its expression is limited to type II fast muscle fibres [19]. The mutation of the gene is due to C-T nucleotide

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transition in exon 16, leading to the replacement of arginine (577R) with a stop codon (577X) leading to premature termination of α -actinin-3 synthesis [1]. The resulting polymorphism of ACTN3 gene is presented in three genetic variants (RR, XX and RX). It has been reported that RR genotype and R allele are associated with power/sprint performance and strength in various sports and different ethnic groups [3, 7-9, 17, 20-23]. Several studies have pointed out that elite power/sprint athletes (jumping, throwing, 100m runners and soccer players rarely exhibit XX genotype [5, 14, 24, 25]. The function of X allele is not fully understood. The XX genotype and X allele may alter the metabolism of muscle cells in aerobic direction resulting in poorer sprint/power performance [20, 21]. However, a handful of athletes with the RX genotype have shown excellent results in power/sprint events [22]. Recently, the research has been focused on the influence of ACTN3 R-power allele dominant (RR + RX) model alone [22, 24] or in combination with other genes [16, 26].

The AMPD1 polymorphism is a nonsense (C34T) mutation in exon 2 resulting in AMPD enzyme deficiency [27, 28]. The AMPD1 gene is presented in three genotypes (CC, TC and TT). The AMPD enzyme catalyses the deamination of AMP (adenosine monophosphate) to IMP (inosine monophosphate), thereby reducing the accumulation of ADP (adenosine diphosphate) and shifting the balance of the myokinase reaction towards ATP generation [6]. The AMPD1 gene is an important regulator of cellular energy metabolism during high-intensity physical exercise [13, 21]. Individuals with the unfavourable mutant TT genotype might show diminished exercise capacity and cardio-respiratory response to exercise [7]. The CC genotype and C allele are considered to be related to performance in power-oriented athletes, and T allele might be a negative factor for athletic performance [29]. However, it was reported that high intensity anaerobic performance was not influenced by AMPD1 genotypes [13].

In various sport disciplines, despite the specific energy demands of a particular sport, an objective assessment of the individual's physical fitness (aerobic and anaerobic capacities) is therefore a necessity for planning purposes [7, 25, 30-32]. Training regimens and conditioning have to be specifically designed and focused on the achievement of maximal responses in the most contributing physical and physiological characteristics in contemporary sports [10, 33].

The potential for achieving maximal performance under anaerobic conditions in power/sprint events is determined by the genetic endowment, properties of muscle fibres, the neuromuscular facilitations and energy metabolism [9, 34, 35]. The Wingate test is one of the gold standards and one of the most popular laboratory protocols for assessment of anaerobic capacity [25, 31, 33, 34, 36]. Research data about its relationship with genetic traits is scarce [13, 24, 37].

The rationale for the study is to probe this insufficiently investigated topic taking into consideration that both ACTN3 and AMPD1 genes play significant contributory role in various anaerobic power-linked sport performances.

The purpose of this study was set in two folds: (1) to compare ACTN3 and AMPD1 genotypes and genotype combinations of Bulgarian athletes with a control group of unrelated healthy individuals; (2) to identify the following:

- Any possible association of the ACTN3 and AMPD1 genetic variants;
- Any potentially beneficial genotype combinations;
- Any R-(RR+RX) and C-(CC+CT) power allele dominant models related to the anaerobic capacity of the athletes performing Wingate test.

2. Methods

2.1 Subjects

Two groups of participants (athletes and controls) were recruited for the study. All subjects received details on the study during an information meeting

and signed an informed consent form. Experimental procedures were conducted in accordance with the Helsinki Declaration for Ethical treatment of Human Subjects. The study was approved by the Ethic Committee of the Research Board of the National Sports Academy, Sofia and by the Ethic Committee of the National Genetic Laboratory, Medical Academy, Sofia. All participants completed a health screening questionnaire via an interview with a medical doctor. Physical examination of all participants was conducted; blood pressure, heart rate and ECG were recorded at rest. The exclusion criteria included irregularities in ECG and blood pressure, history of chronic diseases, current infection, use of antibiotics and herbal, antioxidant or steroid containing supplements.

The group of athletes consisted of 52 Bulgarian male athletes (age: 21.3 ± 1.5 years, stature: 179.3 ± 1.9 cm and weight: 76.6 ± 1.8 kg). They were elite and sub-elite athletes [1] that belonged to the Bulgarian national teams and were qualifiers and participants at international level competitions. The recruited subjects represented a mixed group of athletes competing in various sports such as power events (long and triple jump, javelin throw), power/sprint orientated events (running: 100, 200, 400 m, swimming: 200, 400 m, sprint cycling, boxing, wrestling) and power/sprint-endurance games (soccer, volleyball, handball). The events were stratified on the basis of the relative anaerobic/aerobic energy system contribution, the duration and intensity of the competitive exercise performance in each sport [38].

The control group comprised of 109 unrelated healthy volunteers (male students with very low level of leisure physical activities), matched for (age: 20.6 ± 1.9 years, stature: 175.5 ± 2.8 cm and weight: 72.5 ± 2.3 kg). The students were asked to complete a questionnaire about their physical activity habits.

2.2 Experimental Approach

2.2.1 Genotyping

All participants in the current study were Caucasian

Europeans. Venous blood samples (10 mL) were obtained from all individuals at rest in EDTA (ethylenediaminetetraacetic acid) anti-coagulant vacutainers for genome DNA isolation from white blood cells using a reagent kit Macherey, Nagel, Germany. Genotyping for detecting the ACTN3 R577X variants (rs 1815739) was performed [39] by using forward 5'-CTGTTGCCTGTGGTAAGTGGG-3' and reverse 5'-TGGTCACAGTATGCAGGAGGG-3' primers (Tib Molecular Biology, Germany) and RFLP (restriction fragment length polymorphism) technique [39]. The PCR amplification (Quanta Biotech QB96 Server Gradient Thermocycler) was performed by 35 cycles of denaturation at 95 °C, annealing at 65 °C and extension at 72 °C. The amplified fragment subsequently underwent digestion by Ddel enzyme (NEB, UK). The digested products were electrophoresed in 3% (w/v) agarose gels and visualised by ethidium bromide staining.

The genotyping of the AMPD1 variants (rs 17602729) was performed using a detection method for C34T mutation [28]. The following primers were used: forward 5'-CTCTGACAAATGGCAGCAAA-3' and reverse 5'-TGTCTACCCCAAAGCAGTGA 3' (Tib Molecular Biology, Germany). PCR was carried out for 30 cycles at 94 °C denaturing temperature, 64 °C annealing temperature and 72 °C extension temperature. Ethidium bromide staining of 3% (w/v) agarose gels was done after the digestion with HpyCH4 IV enzyme (NEB, UK).

After completing the stage of genotyping, the results were analysed. ACTN3 and AMPD1 genotype distribution in both groups was found to be in Hardy-Weinberg equilibrium. No statistically significant differences were observed between the groups. Therefore, to further explore the potentially beneficial genetic traits that might be related to attaining higher anaerobic performance, we tested only the group of athletes by using Wingate test.

2.2.2 Wingate Test Protocol

The Wingate protocol consisted of 30 seconds maximal effort on a mechanically braked, pan-loaded Monark 828E cycle ergometer (Monark, Varberg, Sweden). Resistance was set up at 0.075 kg/kg body mass [34] and was preloaded onto weight pan for immediate application at the beginning of the test. The subject's feet were firmly strapped to the pedals, and the seat height was adjusted for optimal comfort and pedaling efficiency. All participants were previously familiarised with the test. The protocol began with 5 minutes warm up period with light cycling resistance and 5 seconds of sprint cycling at the end of every consecutive minute. After 2 minutes of recovery period (very low resistance cycling), 15 seconds of acceleration at 70 rpm with 1/3 of the work resistance was used. Afterwards the full load was applied and the electronic revolution counter activated. Power output for each second was recorded during the 30 seconds period. A computerised system was used to determine the peak power output (P_p) in watts per kg body weight (P_p W kg⁻¹). On the basis of the total work accomplished in 30 s the anaerobic capacity or mean power (P_m) for period of 30 s was calculated in watts per kg body weight (P_m W kg⁻¹).

2.2.3 Statistical Analysis

Differences in genotype distributions, allele frequencies, and associations of genotypes and genotype combinations with the Wingate parameters

(peak power output and mean power) were evaluated by GenStat Discovery Edition 3 and by ANOVA with post hoc Bonferroni test. The Wingate test variables were categorised according to their median. After ANOVA, the associations were examined using Chi2 maximum likelihood test and Fisher's exact test. The Wingate test parameters were expressed in mean value and standard deviation (s). Statistical significance was accepted at $P < 0.05$. The statistical power of the study was calculated post hoc using a statistic power calculator [40] for alpha (α) error level criterion set at 0.05 or 5% confidence level (5% chance the null hypothesis to be rejected incorrectly).

3. Results

The distributions of ACTN3 and AMD1 genotypes and allele frequencies in athletes and in the control group are shown in Table 1. No significant differences in ACTN3 genotype distribution and allele frequencies were found between the athletes and controls. In both groups, the prevalence of R allele (61.5%-64%) was notable (Table 1). No differences in the R-allele power (RR + RX vs. XX) dominant model were found between the athletes ($n = 44$, 84.6%) and the controls ($n = 95$, 87.2%).

In AMPD1 genotype distribution, a null TT genotype was found in athletes, whereas in the control group, the frequency of TT genotype was 1.8% (Table 1).

Table 1 Distribution of genotypes and allele frequencies (%) of ACTN3 and AMPD1 genes in athletes and in the control group.

Gene	Genotype						Allele frequency	
	RR		RX		XX		R	X
Groups	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	%	%
Athletes ($n = 52$)	20	38	24	46%	8	16	61.5	38.5
Controls ($n = 109$)	45	41.2	50	46%	14	12.8	64	36
AMPD1 groups	CC		CT		TT		C	T
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	%	%
Athletes ($n = 52$)	38	73	14	27%	0	0	86.5	13.5
Controls ($n = 109$)	80	73.2	27	25%	2	1.8	86	14

Table 2 Distribution of genotype combinations (%) of AMPD1 and ACTN3 genes in athletes and in the control group.

Groups	RR/CC		RR/CT		XX/CC		XX/CT		RX/CC		RX/CT		RR/TT		RX/TT	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Athletes (n = 52)	17	33	3	6	4	8	4	8	17	33	7	12.0	0	0	0	0
Control (n = 109)	33	30	9	8.3	9	8.3	3	2.8	39	36	14	12.8	1	0.9	1	0.9

No differences in CC and CT genotypes were found between the groups. The prevalence of CC genotype (73%-73.2%), high frequency of C allele (86%-86.5%) and low frequency of T allele (13.5%-14%) was observed in both groups. With regards to the C-allele power dominant model (CC + CT vs. TT), all athletes displayed 100% (n = 52; CC + CT genotype) compared to 98.2% (n = 107) in the controls.

The ACTN3 and AMPD1 genotype combinations in the athletes and the control group are presented in Table 2. Both groups displayed similar combinations of RR/CC (30%-33%), RX/CC (30%-36%), RX/CT (12%-12.8%), XX/CC (8%-8.3%) and RR/CT (6%-8.3%) genotypes. No significant differences between the two groups were found. However, in athletes, the null RR/TT and RX/TT genotypes should be noted.

The results of Wingate test parameters of athletes are shown in Figs. 1-4. Higher (P < 0.05) peak power output (Fig. 1) was found in athletes with RX (P_p = 10.03 ± 0.60 W kg⁻¹) and CT (P_p = 10.30 ± 0.54 W kg⁻¹) genotypes. The association tests showed that values above the median are associated with 75% of RX genotype (P = 0.006) and 71% of CT genotype (P = 0.011), respectively.

Athletes with RX/CT genotype combination (Fig. 2) displayed a significantly higher (P < 0.05) peak power output of 11.10 ± 0.86 W kg⁻¹ when compared to RR/CC, RX/CC, RR/CT and XX/CT combinations. The lowest value of 8.83 ± 0.99 W kg⁻¹ was found in XX/CC combination. Athletes with RX/CT genotype combination showed the highest (P < 0.001) peak power output compared to the average value of P_p = 9.41 ± 0.82 W kg⁻¹ derived from all other genotype combinations pooled together. P_p values above the median are associated with 71% of RX/CT combination (P = 0.030). In addition, the P_p of RX/CT

combination was significantly higher (P < 0.05) compared to R-allele power dominant model (P_p = 9.92 ± 0.52 W kg⁻¹) and C-allele power dominant model (P_p = 9.90 ± 0.50 W kg⁻¹). No significant differences were observed in the mean power (Fig. 3) among ACTN3 and AMPD1 genotypes. When investigating the six genotype combinations (Fig. 4), the results showed that athletes with RX/CT combination have significantly higher (P < 0.05) P_m = 8.93 ± 0.61 W kg⁻¹ when compared to RR/CC and RR/CT genotype combinations. P_m values above the median are associated with 75% of RX/CT genotype combination (P = 0.032).

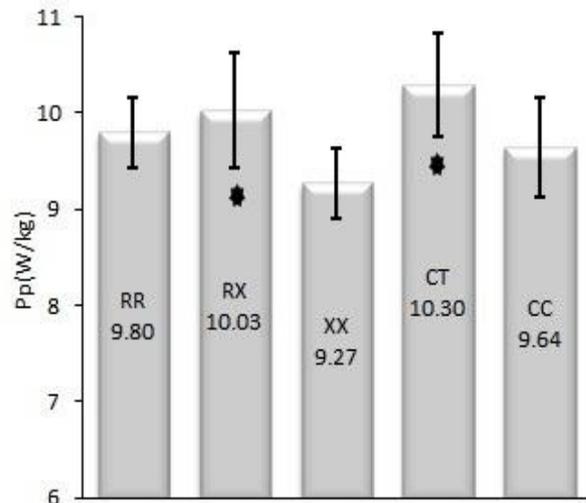


Fig. 1 Peak power output (P_p W kg⁻¹) of ACTN3 and AMPD1 genotypes in athletes.

*P < 0.05—P_p values of RX and CT genotypes versus XX and CC genotypes.

Study power alpha (α)—RX genotype (95%) and CT genotype (99.6%) versus XX genotype and CT genotype (98.2%) versus CC genotype.

Association tests:

P = 0.006—P_p values above the median associated with 75% of RX genotype.

P = 0.011—P_p values above the median associated with 71% of CT genotype.

ACTN3 and AMPD1 Polymorphism and Genotype Combinations in Bulgarian Athletes Performing Wingate Test

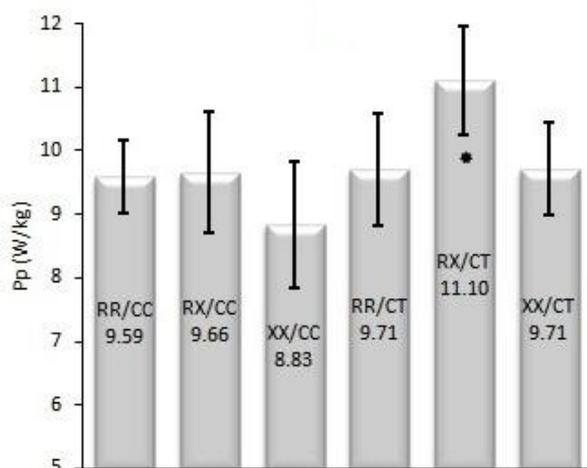


Fig. 2 Peak power output (P_p W kg⁻¹) of *ACTN3* and *AMPD1* genotype combinations in athletes.

* $P < 0.05$ —RX/CT genotype combination versus RR/CC, RX/CC and XX/CC genotype combinations

Study power alpha (α)—RX/CT combination versus RR/CC ($\alpha = 98.7\%$), RX/CC ($\alpha = 95\%$), XX/CC ($\alpha = 96.9\%$), RR/CT ($\alpha = 63.5\%$) XX/CT ($\alpha = 81.2\%$).

Association test: $P = 0.030$ — P_p values above the median associated with 71% of RX/CT genotype combination.

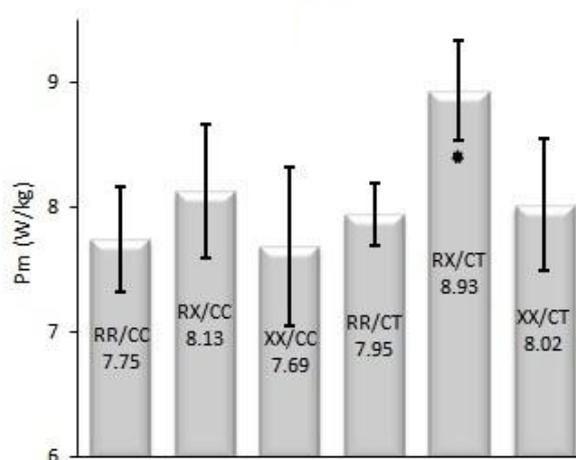


Fig. 4 Mean power (P_m W kg⁻¹) of *ACTN3* and *AMPD1* combinations in athletes.

* $P < 0.05$ — P_m values of RX/CT genotype combination versus RR/CC and RR/CT combinations.

Study power alpha (α %)—RX/CT combination versus RR/CC ($\alpha = 98.5\%$), RX/CC ($\alpha = 75.2\%$), RR/CT ($\alpha = 95\%$), XX/CT ($\alpha = 64.4\%$) and XX/CT ($\alpha = 50.4\%$) genotype combinations.

Association test: $P = 0.032$ — P_m values above the median associated with 75% of RX/CT combination.

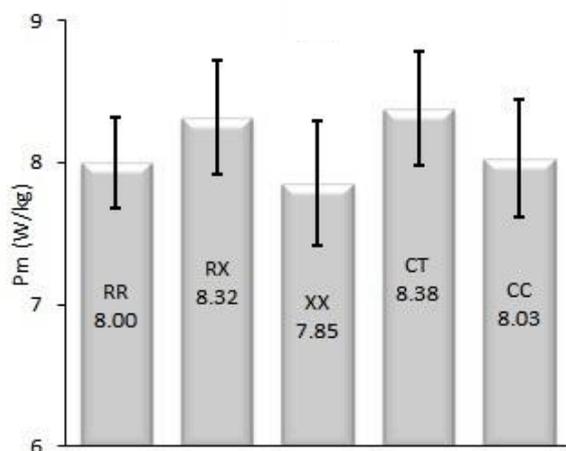


Fig. 3 Mean power (P_m W kg⁻¹) of *ACTN3* and *AMPD1* genotypes in athletes. No significant differences.

4. Discussion

In this study, the highest peak power obtained during Wingate test were found in carriers of RX/CT genotype combination compared to all other combinations and to the carriers of R and C power allele dominant model. A null TT genotype and low T

allele frequency were observed in athletes.

The Wingate test peak power results are in accordance with our previous investigations of male Polish elite athletes of the national basketball, volleyball, handball, rugby and soccer teams [31] and with the data reported in male athletes (track, cycling, soccer, boxing, wrestling, water polo, lacrosse and American football) from Division 1, US Air Force Academy [36].

We expected athletes with RR/CC combination to have the highest anaerobic performance, taking into consideration that R and C alleles are both associated with power/strength and sprint events. In the present study, the unexpected finding was that only 12% of the athletes who carried RX/CT genotype combination have shown higher anaerobic performance. Therefore, the small sample size of this subgroup was of major concern when analysing the results. However, despite the small percentage of the carriers of this genetic trait, our findings were supported by the calculation of the statistical power of the study, the association tests and

the comparison with the R and C power allele dominant models. The association of CT and TT genotypes with anaerobic performance is not clear. It was reported that the Wingate test results are unaffected by AMPD1 genotypes [13]. In contrast, a faster power decrease and a lower mean power was demonstrated in individuals with TT and CT genotypes during the 30 s cycling test [27]. However, despite the 10% decline in the mean power, a better circulatory adaptation to exercise was found in TT and CT individuals with diminished muscular AMPD enzyme activity. It was suggested that it is probably due to an AMPD1 genotype-dependent increase in adenosine, which is known to be an important vasodilator [6]. Our findings suggest that C34T mutation might play an important role in energy metabolism by effecting both AMPD's and AMPK's enzyme activities [13, 21], thereby altering ATP and AMP cellular levels and the anaerobic performance.

Our data about ACTN3 genotype distribution in athletes and controls are in agreement with the findings established by numerous other studies of Caucasian Europeans [5, 11, 14, 17]. Our results confirmed that peak power was not different among ACTN3 genotypes, and support the finding that peak power was significantly higher in the R-allele dominant model as it was found in Japanese athletes [24].

The evidence for beneficial effects of RR genotype and R allele has been provided by numerous studies of mixed sprint/power cohorts of athletes in various sports (sprints, swimming, skating, gymnastics, track and field, throwing, weightlifting, soccer, basketball, volleyball, cricket) [1, 7, 8, 11, 17, 21, 30]. In contrast, the role of XX genotype and X allele on athletic performance was not quite clear. Recent publications have shown that α -actinin-3 deficiency results in a fundamental shift in metabolism from the anaerobic pathway towards the oxidative muscle metabolism and enhanced endurance performance [2, 9].

The expression of the skeletal muscle α -actinin-3

protein is almost exclusively restricted to fast twitch (type II) muscle fibres, where it constitutes one of the major components of the Z-disk. The α -actinin-3 stabilises the muscle contractile apparatus, which may confer a higher capacity for force absorption/transmission compared to type I fibres [22, 37]. The protein interacts with potassium channel proteins, glycolytic enzyme fructose—1,6-biphosphatase, glycogen phosphorylase and the calsarcins which bind to and regulate the expression of calcineurin. This protein is a signalling factor which plays a role in the specification of muscle fibre type and other signalling cascades on the cell membrane of the working muscle [19].

In our study, the simultaneous display of R and C alleles related to power/sprint and the mutant X and T alleles in RX/CT genotype may indicate that all contribute to enhanced anaerobic performance

The limitation of this preliminary study is the small sample size of the group of athletes. Bulgaria is a small country and limited numbers of elite and top athletes in each of the sport disciplines are available. Other studies have also recruited small number of athletes when investigating the influence of genotypes on Wingate test [33] and explosive leg muscle power [7]. The other limitation is that the inexperienced volunteers from the control group could not be exposed to Wingate due to the risk factors related to the metabolic and performance demands of the test.

Experimental studies recruiting large cohorts have to be carried out to validate our current exploratory results. More polygenic profiles should be probed to consider the optimization of training regimen and focussed training interventions to achieve better trainability [10, 18]. Our study addresses the need of exploring the influence of selected genotype combinations and power allele dominant models on the anaerobic capacity.

The findings of this study are applicable in the contemporary sports practice by implementing interpretations of individual genetic profiling and

Wingate test results, leading to personalized training programme. Athletes who are carriers of R- and C-power dominant model are likely to achieve improvement of strength, speed and power training. Carriers of X and T allele should be more realistic about their speed/power potential. Athletes with TT genotype might have limited ventilatory adaptation to higher intensity exercises. Genetic testing on sport participation [3] and talent selection could be appropriate to aspiring young athletes towards training and competing in the most suited sport discipline.

5. Conclusion

The purposes of this study were: (1) to compare ACTN3 and AMPD1 genotypes and genotype combinations of Bulgarian athletes with a control group of unrelated healthy individuals; (2) to identify any possible association of the ACTN3 and AMPD1 genetic variants; any potentially beneficial genotype combinations; any R-(RR + RX) and C-(CC + CT) power allele dominant models related to the anaerobic capacity of the athletes performing Wingate test.

The low frequency of T allele in Bulgarian athletes might indicate that this mutant allele is related to the physical performance. The prevalence of R ACTN3 allele and C AMPD1 allele suggests that they could contribute to anaerobic performance. Higher peak power output in Wingate test is associated with RX/CT genotype combination and R- and C-power dominant models.

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